Isolation of a Soluble Elastin from Lathyrctic Chicks

By BRYAN C. SYKES and S. MILES PARTRIDGE
*Agricultural Research Council Meat Research Institute,
Langford, Bristol BS18 7DY, U.K.*

(Received 2 October 1972)

Elastin is a major structural component of vertebrate blood-vessel walls and is characterized by its insolubility and a unique amino acid composition comprising over 90% non-polar residues. Both the insolubility of fibrous elastin in all systems that do not break peptide bonds and its long-range elastic extensibility in the water-swollen condition are attributed to a structure consisting of large sections of randomly kinked peptide chains interlinked by stable covalent bonds (Partridge, 1962). Cross-link synthesis in elastin and in collagen involves oxidative deamination of lysine residues, and this reaction is inhibited by copper deficiency and also by lathyrism induced by β -aminopropionitrile (for reviews see Partridge, 1968, 1969; Bailey, 1968; Piez, 1968). Experimental lathyrism leads to the accumulation in the tissues of un-cross-linked collagen monomers (Levene & Gross, 1959), and the present study was carried out in the hope of finding a soluble elastin monomer under similar circumstances.

Day-old cockerels of a laying breed (White Leghorn $\times$ Rhode Island Red) were obtained from a local hatchery and raised on a commercial diet supplemented with β -aminopropionitrile fumarate to 0.1% (w/w). After 6 days the chicks began to show signs of lathyrism, including swollen hocks, leg weakness and diminished weight gains, and by 4 weeks fatal rupture of the aorta had accounted for 90% of the birds.

The innominate arteries and aortae of birds from 6 to 10 days old were dissected free from adhering tissue, frozen in liquid N_2 and kept frozen until extraction. Histological examination of the aortae showed swollen walls and disaggregation of the elastic fibres compared with controls. All subsequent operations with the exception of rotary evaporation were carried out at 5°C with pre-cooled reagents. Organic solvents were redistilled analytical grade. The aortae were homogenized in 0.02M-formic acid (20 ml/g wet wt. of tissue) to which was added β -aminopropionitrile fumarate to 0.1 mM. The pH was adjusted to 2.80 with 0.5M-formic acid and the mixture was stirred overnight. After centrifuging in the 10 $\times$ 100 ml angle rotor (MSE no. 59596) at 23 000g for 1 h, the pellet was extracted once more with fresh buffer and recentrifuged. The pooled supernatants were dialysed against 0.1M-ammonium formate and 0.1 mM- β -aminopropionitrile fumarate adjusted to pH 5.5 with 2M-formic acid and then concentrated

fourfold with Carbowax 20M (R. A. Lamb, Alpertons, Middx., U.K.). To 2 vol. of concentrated dialysis residue 3 vol. of propan-1-ol was added slowly followed by 5 vol. of butan-1-ol. The additions were made over 5 h. The alcoholic mixture was stirred overnight and then filtered. The solvents were removed by evaporation on a rotary evaporator, the temperature of the flask contents being kept below 15°C. An evaporation rate of about 500 ml/h was achieved by using a vacuum pump (ED 100; Edwards High Vacuum, Crawley, Sussex, U.K.) protected by two liquid- N_2 traps and by cooling the condenser coil with circulating saline at 0°C from a refrigeration unit. The residue was washed three times with 25 ml of cold chloroform and then dissolved in the minimum volume of 0.02M-formic acid, dialysed against the same, and freeze-dried. Then 30 mg of the freeze-dried material was dissolved in 10% (v/v) acetic acid, centrifuged for 30 min at 25 000g to remove suspended particles and applied to a column (1.2 cm $\times$ 30 cm) of Sephadex G-100 previously equilibrated with the solvent. The column was run with 10% acetic acid and monitored at 280 nm with a Uvicord II Absorptiometer (LKB Produkter, Stockholm, Sweden). The fractions corresponding to the peak eluted at the void volume were collected and freeze-dried.

A 1 mg portion of the freeze-dried material was hydrolysed in a sealed tube for 24 h at 110°C with 6M-HCl, and amino acid analysis was performed on a Locarte analyser (Locarte, London S.W.7, U.K.).

Table 1 shows the composition compared with a sample of insoluble aortic elastin from normal chicks prepared by the hot-alkali extraction method of Lansing *et al.* (1952).

Other workers have reported soluble proteins with similar compositions from the tissues of animals after a period of nutritional copper deficiency and extracted by using methods similar to that described above (Sandberg *et al.*, 1971; Roensch *et al.*, 1972).

As a test of homogeneity samples of protein were complexed with sodium dodecyl sulphate and subjected to electrophoresis on polyacrylamide gel by using methods previously described for collagen (Sykes & Bailey, 1971). However, when stained with Coomassie Blue only faint bands developed. Similar resistance to staining after complexing with dodecyl sulphate is also encountered with oxalic acid-solubilized fibrous elastin (Partridge, 1962). In an attempt

Table 1. Amino acid composition of elastins from aortae of lathyrctic and normal chicks

Experimental details are given in the text.

	Amino acid composition (residues/1000 total residues)	
	Lathyrctic-chick soluble elastin	Normal-chick insoluble elastin
Hydroxyproline	12	14
Aspartic acid	9	5
Threonine	13	8
Serine	16	6
Glutamic acid	15	15
Proline	132	130
Glycine	315	368
Alanine	169	163
Cystine (half)	0	0
Valine	182	169
Isoleucine	19	20
Leucine	49	53
Tyrosine	11	13
Phenylalanine	18	20
Desmosine (quarter)	0	8
Lysine	40	4
Histidine	0	0
Arginine	3	6

to get a clearer demonstration of homogeneity the protein was labelled with ^{125}I (Greenwood *et al.*, 1963) and repurified on a small column of Sephadex G-25 (Ceska *et al.*, 1971). The sample was then run in the same track as a mixture of proteins of known molecular weight. After staining with Coomassie Blue, the gel was cut into 1.3 mm slices, the stained markers were located and the radioactivity of each of the slices was counted on a γ -ray scintillation counter (Panax Equipment Ltd., Redhill, Surrey, U.K.). In a separate experiment, simultaneous runs of iodinated and untreated marker proteins did not demonstrate any differences in their mobilities. Fig. 1 shows that the iodinated protein appeared as a single slightly skewed band with a mobility corresponding to a molecular weight in the range 40000–50000.

On the basis of the observations it would seem that there is present in the tissues of lathyrctic animals a soluble protein, apparently homogeneous in terms of molecular weight, with an amino acid composition

Fig. 1. Electrophoresis of iodinated soluble elastin

The radioactivities of fractions from polyacrylamide gel were counted on a γ -ray scintillation counter. The arrows denote the positions of molecular-weight markers: phosphorylase, 100000; pyruvate kinase, 57000; creatine kinase, 41000; yeast phosphoglucosucrase, 28500.

characteristic of elastin, save for an elevated lysine content and the absence of desmosine cross-links.

The work was supported in part by the Nuffield Foundation.

- Bailey, A. J. (1968) *Compr. Biochem.* **26B**, 297–423
 Ceska, M., Pihl, P., Holmqvist, L. & Grossmuller, F. (1971) *J. Chromatogr.* **57**, 145–147
 Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123
 Lansing, A. I., Rosenthal, T. B., Alex, M. & Dempsey, E. W. (1952) *Anat. Rec.* **114**, 555–576
 Levene, C. I. & Gross, J. (1959) *J. Exp. Med.* **110**, 771–790
 Partridge, S. M. (1962) *Advan. Protein Chem.* **17**, 227–297
 Partridge, S. M. (1968) in *Symposium on Fibrous Proteins* (Crewther, W. G., ed.), pp. 246–264, Butterworths (Australia) Ltd., Sydney
 Partridge, S. M. (1969) *Bibl. Nutr. Dieta* **13**, 99–110
 Piez, K. A. (1968) *Annu. Rev. Biochem.* **37**, 547–570
 Roensch, L. F., Savage, J. E. & O'Dell, B. L. (1972) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **31**, 1500
 Sandberg, L. B., Zeikus, R. D. & Coltrain, I. M. (1971) *Biochim. Biophys. Acta* **236**, 542–545
 Sykes, B. C. & Bailey, A. J. (1971) *Biochem. Biophys. Res. Commun.* **43**, 340–345